STUDIES ON THE PANCREATIC SECRETION IN SKATES.

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From the point of view of comparative physiology the skate possesses many features of interest. Animals such as the elasmobranch fishes, which are generally considered to have remained at a lower point of evolution than mammals, present an opportunity of investigating the intermediate stages of functional development of the different organs of the higher forms. An attempt was made in the present study to investigate the pancreatic secretion in skates, since the anatomical relation of the pancreatic gland in these animals affords certain advantages for such experimental study. In addition some observations were made on the distribution and relations of the pancreatic ducts which present certain peculiarities in this animal form.

Anatomical Data.

The pancreatic gland was investigated in three species of skates, namely, *Raja erinacea*, *Raja diaphanes* and *Raja stabuliforis*. The size of the organ varies of course according to the proportions of the animal. In all these the gland is quite compact, showing no tendency to take the diffuse form seen in most of the higher orders of fishes but rather resembling the analogous structure in the higher vertebrates, including man. Indeed the gland is much more compact in the skate than in the rodent type of mammalians. Disregarding minor differences in the shape of the pancreas in the three species examined, one finds that it always consists of two lobes of unequal size connected by a more or less constricted isthmus of pancreatic tissue. The ventral lobe, which is proximal to the intestine, is considerably smaller and lies attached to the groove formed by the junction of the duodenum and the pyloric end of the stomach. The dorsal lobe is much

larger and lies under the stomach. In the excised pancreas it flattens to a certain degree (Fig. 1).

For studying the distribution of the duct system, the ducts

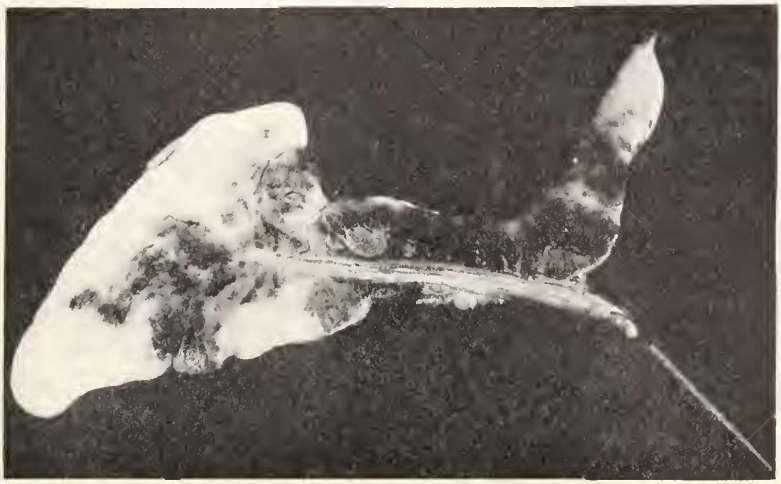

FIG. 1. Pancreatic gland of *R. stabuliforis* injected with carmin-starch-formalin mixture.

were in some cases injected with a mixture of carmin and starch in 10 per cent. formalin, and the fixed preparation was then carefully dissected out. In other cases the ducts were injected with pyroxylin dissolved in acetone and coloured with ultramarine-blue or vermillion. A cast of the duct system was then obtained by digesting away the surrounding glandular tissue by means of a mixture of pepsin and hydrochloric acid. At the Biological Station the mixture was made up by adding a quantity of 0.36 per cent. hydrochloric acid to the gastric mucous membrane excised from skates.

There is usually only one duct connecting the gland with the duodenum. This duct takes the form of a comparatively large tube of approximately uniform caliber, from which smaller ducts are given off. The large duct traverses the dorsal side of the isthmus of the gland and disappears into the midst of the distal lobe. Fig. 1 is a photograph of the pancreatic gland of *Raja stabuliforis* seen from the dorsal side and showing the main duct injected. The length of the fish was 121 cm. The length of the

visible portion of the main duct was 5.8 cm., while the portion connecting the proximal lobe with the duodenum measured 1.8 cm. The diameter of the visible portion of the duct was about 3.5 mm., but it became slightly reduced toward the point where the duct disappeared into the substance of the distal lobe of the gland. Figs. *2a* and *2b* are sketches of the pancreatic ducts of two specimens of *Raja stabuliforis* as they appeared after being injected with the mixture of carmin, starch and formalin. Fig. 3 shows a

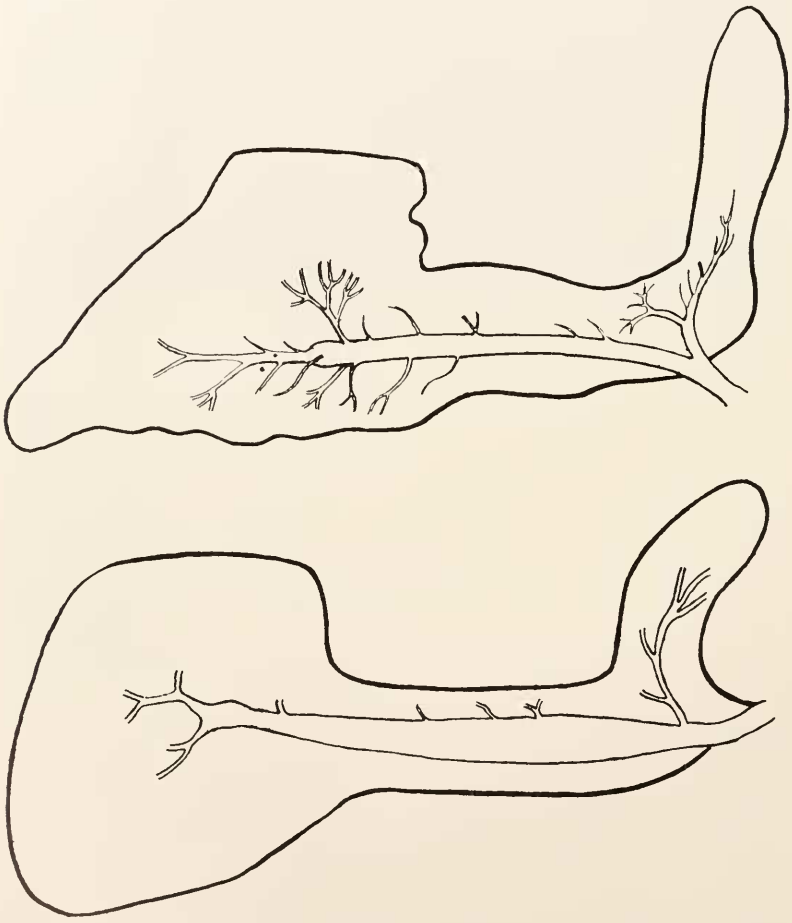

FIG. 2, *a* AND *b*. Sketch of pancreatic ducts of two *R. stabuliforis* injected with carmin-starch-formalin mixture. Only a few secondary, tertiary, etc. ducts could be injected. In case "*a*" the main duct presented a large tube of uniform size. In case "*b*" the main duct was dilated in the middle part.

similar preparation of *Raja diaphanes* and demonstrates the analogous structure of the gland in these two species.

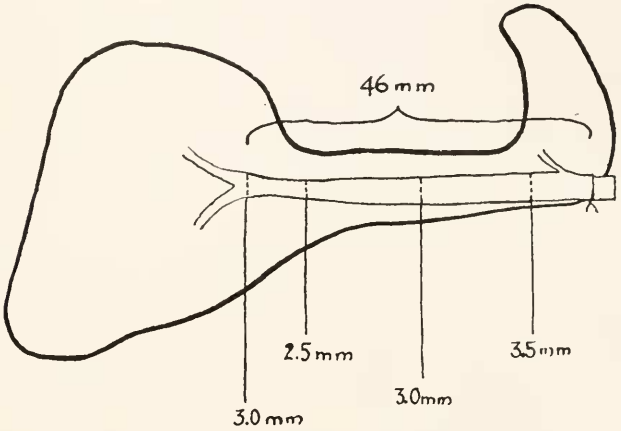

FIG. 3. Sketch of the pancreatic ducts in *R. diaphanes*.

The most striking feature of the duct system in the pancreas of the skate family is the contrast between the calibre of the main duct and that of the secondary ducts. In *Raja stabuliforis*, for instance, the only branch having a fairly large diameter is that which drains the proximal lobe of the gland. All other branches in this species have such extremely small channels that only the very beginning of each could be injected. In *Raja diaphanes* it was found to be very difficult to inject any of the secondary ducts, while in the case of *Raja erinacea* attempts to inject these ducts were entirely unsuccessful.

This peculiar contrast in calibre between the main and the secondary ducts suggests that the latter do not develop as fully in the skate as in higher forms. In mammals we find a gradual diminution in caliber, proceeding from the main duct or ducts through successively smaller divisions until the terminal ductules are reached. In the cat and dog, for instance, even the terminal ductules are of such a caliber that it is comparatively easy to inject the entire system of ducts, as Revell (1) has shown for the pancreas of the dog. On injecting the pancreas of the cat, the writer has observed very thorough penetration of the injection mass into the smallest ductules, but a similar injection could not be made to penetrate the secondary ducts of skates to any extent,

or when it penetrated the cast was so fine that the ducts could not be preserved intact during digestion of the pancreatic tissue.

It is interesting to note that in skates the "Langerhans cells" do not form the regular islands of Langerhans as in mammals. According to Jackson (2), most of the "Langerhans cells" in the pancreatic gland of skates remain in contact with the ducts. They are usually found between the cells forming the outer layer of the ducts. Jackson looks on this peculiar distribution of "Langerhans cells" in the skate's pancreas "not as constituting a fundamental difference as compared with other groups (*e.g.* mammals) but as a more primitive condition, of phylogenetic and ontogenetic interest."

It seems that the whole structure of the pancreas has a more primitive character in skates than in mammals. We are now engaged on a special histological investigation of the pancreatic ducts in the skate. It may be added that the pancreatic ducts are also extremely narrow in some bony fishes, and it is almost impossible to inject them, (Legouis (3), Krüger (4)).

Methods.

Two methods of immobilizing the animals were employed in the present investigation, namely, section of the spinal cord below the medulla and intraperitoneal injection of Dial "Ciba." The first method was based on the investigations of Miss Craw (5), who showed that spinal skates can live for a long time under proper conditions. The operation was performed as follows: A specimen was removed from the water, and its spinal cord was cut quickly below the medulla. A glass tube connected with the sea-water pipe system was inserted into one of the spiracles. If the section of the spinal cord was performed not too near the medulla oblongata respiratory movement continued for several hours. After section of the spinal cord the animal was turned on its back and the abdomen opened. A cannula connected with a graduated tube was then inserted into the pancreatic duct. The common bile duct was ligatured near the duodenum and a cannula connected with a graduated tube was fixed into the gall bladder. Through an opening in the pyloric part of the stomach a glass cannula (for injections into the duodenum) was inserted into the duodenum and tied. In some of the experiments, to prevent fill-

ing of the stomach with sea water, which runs through the spiracles and is sometimes swallowed by the animal, the œsophagus was tied near the cardia. In many of the experiments the rectum was also tied to prevent the escape of the solutions introduced into the duodenum.

In the case of immobilization by "Dial" Ciba (Basel) the animal was taken from the water, placed on its back and held by a wire-net with loops big enough to pass through a hypodermic needle. An injection was then made into the abdominal cavity by means of a syringe with "Dial" 0.35 to 0.40 c. cm. per kilo weight, and the animal put back in the water. In about a quarter of an hour the animal was asleep and underwent the operation described above. The dose of Dial required was much smaller than the corresponding dose for warm-blooded animals (*e.g.* 0.7 c.cm. per kilo weight for a dog or cat). Notwithstanding the skates were perfectly anæsthetized for 24 hours or more with this amount of Dial. Larger doses of Dial stopped the breathing, though it could usually be restored by means of artificial respiration.

The reaction of the pancreatic juice, bile and gastric contents was tested with litmus paper, and whenever possible the hydrogen ion concentration was determined by the colorimetric method.

The proteolytic activity of the pancreatic juice was determined by means of digestion of fresh calf's fibrin, preserved in glycerin and washed thoroughly before the experiment with running water. Protrypsin was activated by an extract of the intestinal mucous membrane of the skate.

The diastatic power of the juice was tested with 1 per cent. soluble starch solution (iodine and Fehling's tests).

The lipolytic power of the juice was determined according to the method of Anrep, Lush and Palmer (6).

The methods of preparation of the different extracts for enzyme determinations will be described later.

Elasticity and contractility of the Main Pancreatic Duct.

Before presenting data concerning the pancreatic secretion the properties of elasticity and contractility of the main pancreatic

duct will be discussed. These properties of the duct determined some special measures employed during the collection of the pancreatic juice. It could be seen very often in the course of an experiment that the fluid in the graduated tube instead of moving forward moved backwards a few divisions. Slight massage of the duct propelled the juice along the tube.

This backward movement of the fluid may sometimes be observed at the beginning of an experiment in spinal animals in quite good condition, but it is seen very often at the end of an experiment when the animal is dying or even shortly after death. Cutting the spinal cord too close to the medulla, which is usually followed by difficulty in respiration, has the same effect. In skates anæsthetized with Dial the contraction of the duct was not at all marked. Thus the main duct possesses a tone of its own, which may be increased or diminished under certain conditions. That we are not dealing with mere elasticity of the walls is shown by the following experiment:

Exp. Aug. 12. R. diaphanes. Spinal preparation. The pancreatic cannula and the graduated tube were filled with filtered sea water containing 0.5 per cent. of urea. The freezing point of this fluid was equal to $-1.89°$ C. The $\triangle$ of the blood of *R. diaphanes* is equal to $-1.80°$ C. (For these determinations I am indebted to Mr. A. F. Chaisson, who worked at the St. Andrews Biological Station.) This fluid was an indifferent one for the tissue of the skate.

From 10 A.M. to 10:30 A.M. the fluid moved from division 139 to division 140 of the graduated tube (1 division). At 10:30 A.M. the graduated tube was turned upright, and the level of the fluid sank very rapidly to 107 (33 divisions). The graduated tube was then closed and the rubber tube connecting it with the pancreatic cannula was twice gently compressed. As a result of this more fluid entered the gland, so that the fluid in the cannula moved up 36 divisions and now stood at 71. When the graduated tube was opened the fluid rose in it to 79 (8 divisions) and in five minutes fell again to 75 (4 divisions). Fifteen minutes later the level was at 74, and some fifteen minutes after that at 73. When the tube was placed horizontally, the fluid moved along it 25 divisions *i.e.*, reached the 98th division.

It may be seen from this experiment that sudden distention of the duct stimulated it to contract. Later it relaxed. When the pressure on the walls of the graduated tube was diminished by placing it horizontally, the fluid moved along 25 divisions. This phenomenon must be ascribed to the elasticity of the duct. Some of the fluid pressed into the gland did not return, being absorbed, or as seems more probable, remaining in the small ducts or in the interstitial tissue.

The preliminary histological investigation of the pancreatic gland in skates, performed in our laboratory by Dr. D. J. Bowie, showed that in the vicinity of the main pancreatic duct there are smooth muscular fibers.

In connection with these findings a method of very gentle pressure on the main duct during the secretory periods was adopted. This ensured that all pancreatic juice secreted during a certain time passed into the cannula and the graduated tube.

Pancreatic Secretion.

A spontaneous pancreatic secretion was noticed in almost every case. In the experiments the secretion in animals with an empty stomach and duodenum was very scanty. In *R. diaphanes* it averaged only 0.02 c.cm. in one hour. Sullivan (7) has attempted to collect the pancreatic juice in *Carcharias littoralis* over several days. Through an incision in the abdomen a glass cannula was inserted and fastened in the central end of the pancreatic duct. To the outer end of the cannula a small sterilized balloon was attached. Although there was great difficulty in keeping such fish alive, the operation was successful in six cases. The quantity of juice thus collected by Sullivan was as a rule small, however, and it had no digestive activity.

The narrowness and fragility of the pancreatic duct in *Scyllium catulus* and *Lamna cornubica* according to Yung (8) make the preparation of a fistula in the living animals impossible.

Attempts were made to stimulate pancreatic secretion by the following methods: (1) Introduction into the duodenum of HCl solution of different concentrations (0.36 per cent. to 0.95 per cent.). (2) Introduction of a mixture of equal parts of 0.36 per cent. HCl + 2 per cent. urea solution. (3) Intravenous injection of secretin (a cannula was inserted in the central end of one of the gastric veins; secretin was prepared in the usual manner by the action of 0.36 per cent. HCl on the mucous membrane of the duodenum and of the spiral intestine of the skate). (4) Injection of pilocarpin hydrochloride solution intravascularly and into the ducts of the pancreas.

The introduction of HCl solution into the duodenum usually had a definite positive secretory effect. 0.49 per cent. HCl solution was more effective than 0.36 per cent. and 0.96 per cent. solutions.

In some cases the action of the hydrochloric acid was very insignificant. This was usually the case in fasting animals. On the other hand, the introduction of an acidified peptone solution or of an acid digest of fish muscle increased the subsequent secretory effects of HCl solution. In the experiment quoted below (Experiment of July 23) 0.49 per cent. HCl injected into the duodenum after an acid digest, gave the greatest secretion of pancreatic juice ever observed in these experiments.

Exp. July 23. R. diaphanes. ♂. Weight 4416g. 1.5 c. cm. Dial injected at 8:15 A.M. Operation from 8:40 A.M. to 9 A.M. Stomach contained a small amount of food. Duodenum cannulated. Tying of the pylorus produced vomiting, also observed in several previous experiments. Pancreatic duct cannulated. The secretion was noted in the divisions of the graduated tube every 30 minutes.

Time.	Secretion in Divisions.
9 A.M. to 9:30 A.M.	0.5
9:30 A.M. to 10 A.M.	4.5
10 A.M. to 10:30 A.M.	2.0
10:30 A.M. 50 c. cm. of digest[1] injected into the duodenum	
10:30 A.M. to 11 A.M.	5.5
11 A.M. to 11:30 A.M.	2.5
11:30 A.M. to 12 noon	8.5
12 noon to 12:30 A.M.	7.5
12:30 P.M. to 1 P.M.	7.0
1 P.M. to 1:30 P.M. / 1:30 P.M. to 2 P.M.	10.0
2 P.M. to 2:30 P.M.	4.0
2:30 P.M. 50 c. cm. of digest injected into the duodenum. The fluid was partly evacuated from the anal opening.	
2:30 P.M. to 3 P.M.	3.5
3 P.M. to 3:30 P.M.	3.5
3:30 P.M. to 4 P.M.	3.0
4 P.M. to 4:30 P.M.	3.0
4:30 P.M. Rectum tied. 50 c. cm. of 0.49 per cent. HCl injected into the duodenum.	
4:30 P.M. to 5 P.M.	3.0
5 P.M. to 5:30 P.M.	12.0
6 P.M. to 6:30 P.M.	13.0
6:30 P.M. to 7 P.M.	11.0
7 P.M. to 7:30 P.M. / 7:30 P.M. to 8 P.M.	16.0
8 P.M. to 8:30 P.M.	10.0
8:30 P.M. to 9 P.M.	7.0

[1] The digest was prepared as follows: Two stomachs of skates and skate's muscles were mixed July 22 with 0.47 per cent. HCl solution and placed in the incubator (10 A.M.). July 23 (9 A.M.) almost all was digested. The fluid acquired a brownish colour. pH of this fluid was 4.2.

Experiment stopped. Animal was in good condition. Amount of pancreatic juice secreted during 12 hours 0.55 c. cm. Its pH = 7.2. Weight of the pancreatic gland 5.35 g. Content of the duodenum alkaline on litmus. Stomach contained water and mucus only, reaction slightly acid on litmus.

The last part of the experiment, *i.e.*, the action of 0.49 per cent. HCl solution, is represented graphically in Fig. 4. The quantities of juice secreted are shown in actual volumes in c.cm. The

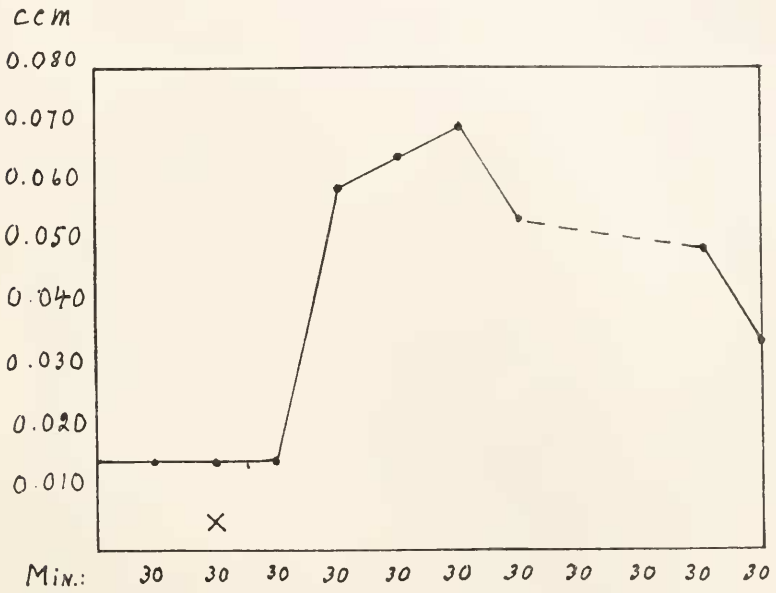

Fig. 4. Curve of the pancreatic secretion in a skate after injection of 0.49 per cent. solution of hydrochloric acid. The ordinates represent the amount of pancreatic juice in c. cm. Every division of abscissa is equal to 30 minutes. At *X* 50 c. cm. of acid was injected into the duodenum.

curve is typical for the action of hydrochloric acid. It is reminiscent of the corresponding curve in warm-blooded animals (dog, man), which generally shows a sharp rise before reaching its peak, and then gradually descends. There is however a stricking difference in the time required for this phenomenon in warm-blooded animals and in the skate. Thus the latent period for the secretory action of HCl in dogs is from $1\frac{1}{2}$ minutes (dogs with a permanent pancreatic fistula) to 4–5 minutes (acute experiments). In skates it requires half-an-hour for the acid to develop its secretory effect. Secretion on 200 c.cm. of 0.5 per cent. HCl in a

dog of 15 to 20 kilos with a permanent pancreatic fistula lasts about an hour and a half to two hours, and somewhat longer in an acute experiment. In a skate of 4 to 5 kilos weight the pancreatic secretion on 50 c.cm. of 0.5 per cent. HCl extends over 4 or more hours. Another feature of the pancreatic secretion in skates is its scantiness as compared with the secretion similarly stimulated in dogs. Thus in the experiment on a skate quoted above the amount of pancreatic juice secreted on 50 c.cm. of 0.5 per cent. HCl during four hours was 0.43 c.cm. In a dog of approximately four times greater weight, a correspondingly greater amount of 0.5 per cent. HCl, *i.e.*, 200 c.cm., in 1 hour, and 45 minutes, gave 138.0 c.cm. of juice (Dolinski (9)). One of the factors responsible for the scantiness of the pancreatic secretion in the skate is its low body temperature as compared with warm-blooded animals. Thus the temperature of the water running through the gils in this experiment was 13.5° C. The water in the tank which was exposed to the air of the laboratory was 15° C., and the temperature of the fish was probably about the same. If Van't Hoff's law could be applied to the secretory processes, namely, that the velocity of a chemical reaction is doubled with each 10° C. rise of temperature, it still could not explain the difference in the activity of the pancreatic gland in warm- and cold-blooded animals. The most probable explanation of the scarcity of the pancreatic secretion in skates is that this organ is not developed to the same degree as in warm-blooded animals. Thus, in dogs, for example, the average weight of the pancreatic gland is approximately $\frac{1}{400}$ of the body weight, whereas in *R. diaphanes* it averages only $\frac{1}{800}$ of the body weight.

No essential difference was noted in the secretory action of the hydrochloric acid alone or when mixed with 2 per cent. urea, one of the permanent constituents of the blood of this fish.

The humoral character of the pancreatic secretion in the skate was emphasized by the secretory action of secretine prepared on 0.36 per cent. hydrochloric acid. The following experiment is quoted as an example:

Exp. July 26. Raja diaphanes. Spinal preparation. Stomach empty. Pan-

creatic duct and common bile duct cannulated. Secretion noted every 15 min. in divisions of graduated tubing.

Time.	Secretion.
3:25 P.M. to 3:40 P.M.	3.5
3:40 P.M. 12:05 c. cm. of secretine injected into one of the gastric veins.	
3:40 P.M. to 3:55 P.M.	2.5
3:55 P.M. to 4:10 P.M.	13.0
4:10 P.M. to 4:25 P.M.	2.0
4:25 P.M. to 4:40 P.M.	3.0
4:40 P.M. to 4:55 P.M.	3
4:55 P.M. to 5:10 P.M.	0

Even after direct introduction into the blood of the secretory agent, *i.e.*, of the secretine, the latent period of secretion was equal to 15 minutes. This fact may probably ve explained by the slowness of the circulation in skates. In the summer time, under the conditions of the experiment, the heart usually contracted only 22 to 26 times in a minute.

The data reported above show that hydrochloric acid in a concentration of about 0.5 per cent. stimulates the pancreatic secretion in skates. The action of the hydrochloric acid is greater after previous introduction into the duodenum of the acid products of protein digestion. The mechanism of the secretory action of HCl is probably humoral, through the formation of secretine.

Experiments with Pilocarpin.

Different kinds of experiments were devised to ascertain the effect produced by pilocarpin on the secretory function of the pancreatic gland, using it as a drug to stimulate the peripheral parts of the parasympathetic nervous system. The following procedures were adopted: (1) Intravenous injection of 0.1 per cent. pilocarpin hydrochloride solution in variable amounts (from 2 to 4 mg. or more). The solution was usually injected with a fine hypodermic needle into one of the gastric veins or into the portal vein itself. (2) Injection of the pilocarpin solution into the conus arteriosus or into the ventricle of the heart. (3) Injection of the solution into the superior mesenteric artery, which supplies with blood the whole dorsal part and half the isthmus of the pancreatic gland, *i.e.* the greater part of the organ. (In sev-

eral experiments the pilocarpin solution was stained with methylene blue.) (4) Introduction of the pilocarpin solution into the pancreatic ducts. For this purpose the graduated tube and the cannula were filled with the solution, and kept in a vertical position for 15 minutes. Under its own pressure the solution entered the duct and distended it. The graduated tube was then returned to the horizontal, and the rate of secretion noted.

In no case did pilocarpin activate the pancreatic secretion or increase spontaneous secretion. Though the negative results in some of the experiments might be explained by the damming back of the pilocarpin solution owing to the narrowness of the small pancreatic ducts, through which the solution could not penetrate to the alveoli, an analogous explanation could not be applied to the experiments where the drug was injected into the arterial system.

As an example of the action of pilocarpin the Experiment of July 30 is quoted.

Exp. July 30. R. diaphanes ♀ Weight 5010 g. 8:15 A.M. Dial injected intraperitoneally. 8:30 to 8:50 A.M. abdomen opened; pancreatic duct cannulated. There was spontaneous pancreatic secretion, which became very insignificant about 1:15 P.M. Skin and muscles covering the heart chamber removed. Pancreatic secretion was noted in divisions of graduated tubing every 15 min. unless marked otherwise.

Time.	Secretion.	Heart Rate Beats per Minute.
1:15 P.M. to 1:30 P.M.	2	
1:30 P.M. to 1:45 P.M.	1	
1:45 P.M. to 2:00 P.M.	0	22
2 P.M. 2 mg. of pilocarpin hydrochloride in 2 c. cm. of distilled water injected into the bulbus aortae. 2.03 P.M. heart 12 beats per minute; 2.06 P.M. heart 18 beats per minute.		
2:00 P.M. to 2:15 P.M.	1	18
2:15 P.M. to 2:30 P.M.	1	18
2:30 P.M. to 2:45 P.M.	1	18
2:45 P.M. to 3:00 P.M.	1	
3 P.M. 2 mg. pilocarpin hydrochloride in 2 c. cm. of distilled water injected into the bulbus aorta. 3.05 P.M. heart 18 beats per minute.		
3:00 P.M. to 3:15 P.M.	0	16
3:15 P.M. to 3:30 P.M.	0	16

3:35 P.M.	4 mg. of pilocarpin hydrochloride in 4 c. cm. of methylene-blue solution injected into the superior mesenteric artery. The main lobe of the pancreatic gland and half the isthmus became blue. Heart rate at 3.40 P.M. 8 per min. Respiratory movements stopped and did not recover till the end of the experiment. Sea water continued to run through the gills. 3:47 P.M. heart rate 14 per min.		
3:35 P.M. to 4:00 P.M.		1	16
4:00 P.M. to 4:15 P.M.		0	8

This experiment shows that pilocarpin introduced into the arterial system in no way stimulates the pancreatic secretion. The heart rate is influenced by pilocarpin, though in far lesser degree than in warm-blooded animals.

Properties of the Pancreatic Secretion.

Although the pancreatic secretion in skates was so scanty (especially in *R. erinacea*), a certain amount of juice (0.2 to 0.5 c.cm.) was obtained in almost every experiment. Pancreatic juice was also collected from the main duct of freshly caught *R. stabuliforis* (in some cases to the amount of 0.5 c.cm.).

The pancreatic juice of the three species investigated is a colourless, almost neutral fluid. The hydrogen ion concentration of the juice determined colorimetrically (Felton's (10) spot method or British Drug Houses Capillator) varied from 6.6 to 7.2 (eleven determinations).

For the determination of its enzymatic activity, the pancreatic juice was usually diluted with distilled water, and the hydrogen ion concentration of the mixture was adjusted to a certain point by means of anhydrous sodium carbonate or corresponding buffer solutions.

These experiments showed that the pancreatic juice of the skate possesses proteolytic, diastatic and lipolytic action. The proteolytic action was increased by adding an extract of the intestinal mucous membrane to the pancreatic juice. The diastatic ferment was effective without any activator. In what form, *i.e.* active or inactive, the pancreatic lipase is secreted one cannot say

since in the method of determination of Anrep, Lush and Palmer (6) sodium glycocholate is used, and this activates the prolipase.

Thus the pancreatic juice of skates contains all three enzymes which are found in the pancreatic juice of higher mammals including man.

To prove conclusively that the pancreatic gland of skates produces these enzymes several experiments were performed with pancreatic extracts. This was the more important since Yung (8) reported that some of the pancreatic extracts of *Scyllium catulus* and *Lamna cornubica* were inactive towards fibrin but always active in the digestion of starch and emulsification of fat. Sullivan (7) could not demonstrate any amylolytic action of water-glycerin extracts of the pancreatic gland of *R. erinacea*.

The pancretic extracts were prepared on 30 per cent ethyl alcohol, the extracts of the intestinal mucous membrane with 0.9 per cent NaCl. They were kept with toluene for several days at room temperature and then filtered through cheese-cloth.

As an example I quote one of the experiments with *R. erinacea*.

Exp. July 22. R. erinacea. The pancreatic gland (weight 1 g.) extracted for three days with 2 c. cm. of 30 per cent. alcohol. July 25, filtered through cheese-cloth. Mucous membrane of the duodenum and of the spiral valve extracted with 0.9 per cent. NaCl for three days.

Pancreatic Amylase.

July 25.	*Control.*
3:50 P.M. 3 drops of pancreatic extract plus 7 drops of 1 per cent soluble starch solution, plus 7 drops of distilled water, plus one drop of toluene, pH = 6.6, in incubator at 37° C.	3:50 P.M. 7 drops of 1 per cent. soluble starch solution plus 7 drops of distilled water, plus one drop of toluene, pH = 6.6, in incubator at 37° C.
7:00 P.M. Reaction with iodine—colorless.	7.00 P.M. Reaction with iodine—blue.
8:00 P.M. Ditto. Fehling distinctly positive.	8:00 P.M. Ditto. Fehling negative.

Pancreatic Lipase.

July 26.	*Control,*
11:00 A.M. 3 drops of pancreatic extract plus 2 c. cm. of buffer solution, pH = 8.0, plus 2 c. cm. of glycerol-triacetate, plus 9 drops of sodium glycocholate solution, plus 6 drops of phenol red, plus toluene.	11:00 A.M. Everything in same proportion, except pancreatic extract which was not added.
8:00 P.M. Still pink.	8:00 P.M. Pink.
July 27.	
8:00 A.M. Yellow (pH = 7.0).	8:00 A.M. Pink.

PANCREATIC PROTEASE.

July 27.		*Control.*
10:15 A.M. 5 drops of pancreatic extract, plus 5 drops of distilled water, plus one drop of phenol red, plus one drop of toluene, pH adjusted with Na_2CO_3 to 8.0 and fibrin added.	10:15 A.M. Ditto, plus one drop of intestinal extract.	10:15 A.M. 5 drops of intestinal extract, plus 5 drops of distilled water, plus one drop of toluene, plus fibrin. pH = 8.0.
12:30 P.M. No change.	12:30 P.M. Completely digested.	12:30 P.M. No change.
8:00 P.M. No change.		8:00 P.M. No change.
July 28.		
10:15 A.M. No change. Added one drop of intestinal extract.		10:15 A.M. No change. Added 5 drops of pancreatic extract.
1:00 P.M. Completely digested.		1:30 P.M. Completely digested.

This experiment shows that the pancreatic gland of *R. erinacea* possesses diastatic, lipolytic and proteolytic action. The pancreatic protease is contained in the gland in the form of zymogen.

Experiments with the alcoholic extracts of the pancreatic gland of *R. diaphanes* gave similar results. There were some differences in the rapidity with which the pancreatic enzymes of *R. diaphanes* acted when compared with the action of corresponding enzymes of *R. erinacea*. Thus in the Experiment of August 2, the alcoholic extract of the pancreas of *R. diaphanes* changed the reaction of glycerol-triacetate mixture from pH = 8.0 at 9:10 A.M. to pH = 7.4 at 3:30 P.M., pH = 7.2 at 6:00P.M., and finally to pH = 7.0 at 7:30 P.M. Since the enzymatic strength of extracts prepared from one and the same species of fish varied in different extracts, these variations are to be attributed more to the mode of extracting than to real difference in the content of enzymes in the gland.

One point is worth mentioning. According to Sullivan (7) the water-glycerin extracts of pancreas of different elasmobranch fishes did not digest either the coagulated protein of Mett's tubes or fibrin. These extracts digested gelatine only after activation with water-glycerin extract or chloroform extract of the duodenal mucous membrane, or the extract of the mucous membrane of the spiral valve, the last being the most effective.

As may be seen from this study all pancreatic extracts of *R. erinacea* and *R. diaphanes*, as well as the pancreatic juice of these two species and of *R. stabuliforis*, after activation with the intestinal extract, digested fibrin rapidly.

To verify Sullivan's statement that the mucous membrane of the spiral valve contains more enterokinase than that of the duodenum, special experiments were performed. Samples of the same pancreatic extract *R. diaphanes* were activated with 0.9 per cent NaCl extracts of the mucous membrane of the duodenum and the spiral valve (also *R. diaphanes*). The results were as follows:

The pancreatic extract 15 drops (diluted twice with water and with pH adjusted to 8.0) did not digest fibrin in 48 hours.

The same pancreatic extract in the same dilution with the addition of 4 drops of duodenal mucous membrane extract, digested fibrin in 11 hours.

The same pancreatic extract in the same dilution, plus 4 drops of spiral valve extract, digested the same amount of fibrin in about 20 hours.

Both intestinal extracts alone were inactive towards fibrin.

Thus the duodenal extract showed greater activating power than the spiral valve extract. This was probably due to the presence of a greater amount of mucus in the latter.

Note on the Secretion of Bile

The special arrangement of the experiments in this investigation (tying of the common bile duct and insertion of a cannula into the gall bladder) made it possible to study the bile secretion. The bile (usually dark or emerald green gall-bladder bile in fasting animals) was pressed out from the viscus. The freshly secreted bile was of a straw-yellow color. The reaction of the gall-bladder bile in *R. diaphanes* was slightly acid (average pH = 6.4). The reaction of the gall bladder bile in *R. erinacea* according to Miss Mackay (11) was in average pH 6.3. The reaction of the hepatic bile was slightly alkaline (pH 7.5 to 7.6). The secretion of bile was slow and scanty, although more copious than that of the pancreatic juice. The average rate of bile secretion in fasting *R. diaphanes*, without the application of any stimuli, was from 0.01 to 0.02 c.cm. in thirty minutes. In a successful long experiment more than 1 c.cm. of freshly secreted bile was obtained. In *R. erinacea* the secretion was much slower, producing on the average 0.01 c.cm. per hour.

Introduction into the duodenum of 0.36 per cent. to 0.49 per cent. HCl solutions, in some cases mixed with bile, as well as 10 per cent. Witte's peptone solution, increased the secretion of bile, sometimes doubling it.

Conclusions.

Although the pancreatic gland of the skate secretes the same enzymes as the pancreas of mammals, it seems that in the skate this organ has not attained to the high stage of development of the mammalian pancreas. This is indicated by the scantiness of the pancreatic secretion in skates, the peculiar arrangement of the secondary pancreatic ducts, marked by their narrowness, and the smaller weight of the pancreatic gland in relation to the body weight as compared with warm-blooded animals.

Under the experimental conditions described the pancreatic secretion in skates is continuous but scanty. The hydrogen ion concentration of the pancreatic juice is equal to pH 6.6 to 7.2. This again is a special feature of the secretion, since the pancreatic juice in mammals (dog, cat, man) is decidely alkaline (average pH = 8.4). Whereas in mammals the pancreatic juice plays an important part in the neutralisation of acid chyme entering the duodenum, in skates the scanty and almost neutral pancreatic secretion cannot be an important factor in this respect. The reaction of the gall-bladder bile in skates is slightly on the acid side, and that of the hepatic bile very faintly alkaline. Nevertheless the reaction in the duodenum is strongly alkaline, this being evidently due to the alkaline secretion of the succus entericus

Hydrochloric acid introduced into the duodenum activates the pancreatic secretion, probably in a humoral way, since intravenous injections of secretine produce a positive secretory effect. Parasympathetic poison, such as pilocarpin, does not influence the pancreatic secretion in any way.

Summary.

1. The system of pancreatic ducts in *R. erinacea*, *diaphanes* and *stabuliforis* presents certain peculiarities which differentiate it from the analogous system of ducts in higher mammalian animals.

2. Pancreatic secretion in skates which have previously fasted

for several days is continuous but very scanty. Hydrochloric acid and secretin increased this secretion. Pilocarpin was without effect. The previous introduction into the duodenum of the acid digest of proteins increased the secretory effect of the hydrochloric acid.

3. The pancreatic juice is a neutral fluid (pH = 6.6 to 7.2), possessing proteolytic, diastatic and lipolytic action.

4. Alcoholic extracts of the pancreas show the same enzyme action as the juice. A proteolytic enzyme is contained in the gland in the form of protrypsin, which may be activated by 0.9 per cent. NaCl extract of the mucous membrane of the duodenum and spiral valve.

5. Bile is secreted continuously. Introduction of 0.36 per cent. HCl solution and 10 per cent. Witte's peptone solution into the duodenum increases the secretion.

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